IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	Confirmation No. 6572
LALVANI et al.	Atty. Ref.: 3772-19
Appln. No. 09/308,725	T.C. / Art Unit: 1648
Filed: January 13, 2000	Examiner: S.B. Chen

FOR: ASSAY METHOD FOR PEPTIDE SPECIFIC T-CELLS

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STATEMENT OF THE SUBSTANCE OF THE INTERVIEW

March 2, 2007

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

With regard to the Interview Summary dated January 31, 2007, the following is Applicants' statement of the substance of the interview as required by the Examiner.

Applicant's representative and the examiner discussed the minor informalities pending in the application, addressing the informalities in our response, and the prior art rejections of record in view of the latest claim amendments. No agreement as to allowable subject matter was reached, but it appeared that progress had been made by the filing of the responses to the Examiner's Office Action.

At the end of the interview, the Examiner described Miyahira et al. (J. Immunol. Meth. 181:45-54, 1995) as being the closest prior art. She suggested the possibility of a new obviousness rejection based on Miyahira et al. alone or in combination with another prior art reference. The "precursor frequency analysis" using "freshly isolated spleen cells" from immunized mice in Miyahira et al. was noted along with the results of the "immediate ELISPOT assay" shown in Fig. 7 (left panel). The undersigned noted that Miyahira et al. argued the validity of their ELISPOT assay for precursor frequency analysis on the basis that its results were similar to those obtained by limiting dilution analysis (LDA) (see also Applicants' discussion in the Amendment filed December 20, 2006 on page 15). The Examiner asked for a citation to support the Amendment's argument, "It was well known in the art that LDA makes no distinction between memory progeny T

cells and effector T cells." Miyahira et al. admit that their ELISPOT assay enumerates precursors of effector T cells (instead of effector T cells themselves). The immediate ELISPOT assay refers to use of fresh spleen cells without *in vitro* culture, but does not enumerate "T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide and are capable of immediate effector function without the need to effect division/differentiation by *in vitro* culture in the presence of the T cell-activating peptide" as required by Applicants' claims. A detailed explanation of the differences between the assay in Miyahira et al. and Applicants' invention follows.

The concept of Limiting Dilution Analysis (LDA) has been established in the field of immunology for many years (e.g., the textbook by Lefkovitz & Waldmann, *Limiting Dilution Analysis of Cells of the Immune System*, Cambridge University Press, 1979). A more recent description of LDA is contained in Janeway et al., *Immunobiology, 6th Ed.*, Garland Science Publishing, 2005), a copy of pages 709-711 is attached. LDA depends for its result on the proliferation of T cells in response to specific antigen, which usually occurs over several days of *in vitro* culture (see page 711). Memory progeny T cells which recognize the antigen would be expected to proliferate under such conditions. Effector T cells which recognize the same antigen would not be expected to proliferate under these conditions, although they would probably remain viable. Following such *in vitro* culture, the CD8+ subsets derived from both cell types should be capable of lysing target cells in a chromium-release assay of the type employed in the art. The attached description of LDA makes clear that several days of *in vitro* culture allows growth and differentiation of T cells. Such a result is prohibited by the pending claims which require enumeration of "T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide and are capable of immediate effector function without the need to effect division/differentiation by *in vitro* culture in the presence of the T cell-activating peptide."

Miyahira et al. compare an ELISPOT assay with LDA using a chromium-release endpoint assay (the Materials and Methods section refers to '*Precursor frequency analysis*' on page 47). The authors conclude that "the number of antigen specific $CD8^+$ T cells detected by both assays was very similar" on page 52, right column, lines 8-9, and that "[the ELISPOT assay] compares favorably with the standard frequency precursor

analysis which uses the limiting dilution technique" on page 53, right column, last line, to page 54, left column, line 2. Since the results of the ELISPOT assay of Miyahira et al. and LDA are comparable, growth and differentiation of T cells that have been pre-sensitized *in vivo* to the T cell-activating peptide must have occurred under the conditions employed by Miyahira et al. Otherwise, the results would not have been comparable because the number of T cells would have been reduced in the absence of their growth and differentiation.

In light of what was generally known at the time the invention was made about the fundamental principle underlying limiting dilution analysis (i.e., antigen-induced cell proliferation) and the Miyahira et al. disclosure, the skilled person would have unambiguously concluded that the ELISPOT protocol described by Miyahira et al. must be measuring a T cell population which proliferated *in vitro* in response to specific antigen. But such proliferation is prevented under the conditions of Applicants' invention.

The Examiner is invited to contact the undersigned if any further information is required to complete the record of the substance of the interview.

Respectfully submitted,

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